West Nile virus transmission

along a forest-to-urban gradient in Maryland and Washington DC

From May through October 2008 we visited 18 sites in Maryland, Washington DC, and Virginia as a part of our project to understand West Nile virus transmission. We continued work at the sites we added in 2007, including Crofton, Millersville, Arlington, Patuxent, and two sites adjacent to (~1-2 km from) our previous sites, N Foggy Bottom, and N Baltimore. The overarching goal of our research is to determine why some areas seem to have more infected birds, mosquitoes, or people than other areas.

However, in 2008, we took our research a significant step forward. Our past findings had suggested that American robins were heavily preferred and fed on by the mosquitoes most important in transmitting West Nile virus (the northern house mosquito, *Culex pipiens*) See past reports for the amazing feeding patterns of mosquitoes in which robins were fed on 5-30 times more likely than we would expect based on their abundance!

We used a mathematical model to show that the strong feeding preference of mosquitoes for robins, combined with the infectiousness of robins in the week after being bitten by an infected mosquito (the length of the infectious period), made robins the most important bird in West Nile virus transmission. In fact, we estimate that 68-88% of infected mosquitoes at four of our sites became infected after feeding on a robin (Figure 1).

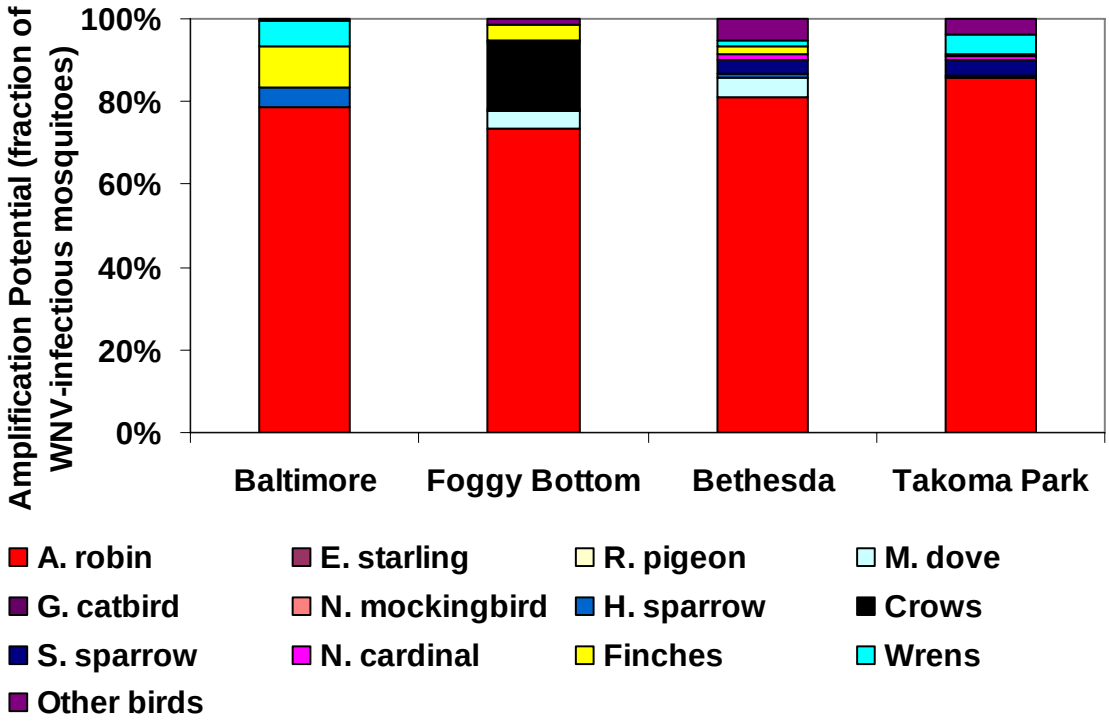


Figure 1. Relative importance of different birds in infecting mosquitoes with West Nile virus, based on mosquito feeding patterns at our sites from 2004-2008, and the infectiousness of different birds based on studies by CDC scientist Dr. Nick Komar. The y-axis indicates the fraction of all infectious mosquitoes that likely became infected from feeding on that bird at that site.

We wondered whether it might be possible to actually decrease West Nile virus transmission by vaccinating American robins. So, we did a few things to prepare for a field test of this idea. First, in collaboration with Dr. Laura Kramer from the New York State department of health and Dr. Jeff Chang from the CDC, we performed a small laboratory study to determine if one of the currently available vaccines (developed by Dr. Chang) might be effective in immunizing robins against West Nile virus. We used a vaccine that has been licensed by the USDA for use in horses and is currently undergoing phase 3 human trials to be licensed for human use as well. The results of our study are shown in Figure 2. This graph shows that the concentration of virus in the blood of vaccinated robins (the solid lines and filled symbols) was always lower than the red line, indicating that these robins would not infect any mosquitoes that fed on them (based on other work – we didn't actually let mosquitoes feed on the birds). In contrast, robins that weren't vaccinated (dashed lines) were infectious to mosquitoes for 3-4 days after being infected with West Nile virus. In addition, the vaccinated birds developed a strong antibody response. Thus, the vaccine was very effective at protecting the robins from the disease, and in making them non-infectious.

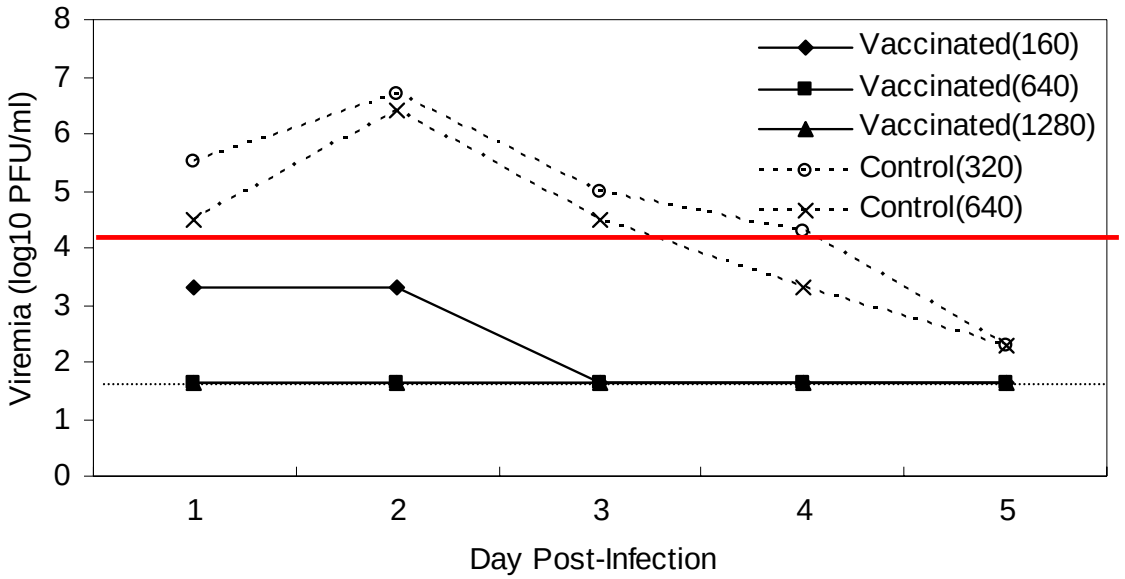


Figure 2. Daily measures of the viremia (the concentration of virus in the blood) on a log scale (so 5 is ten-fold higher than 4 which is ten-fold higher than 3) of three vaccinated and two control hatch year American robins challenged with West Nile virus. The red line shows the minimum viremia below which no mosquitoes would become infected when feeding, based on previous research, and the horizontal dotted line shows the limit of detectability, $10^{1.7}$ PFU/ml (meaning that for points at this level we didn't find any viral particles in the birds' blood, but there could have been a very small number). The numbers in parentheses in the legend gives the antibody titers two weeks post-inoculation – bigger is better, and anything greater than 40 suggests a decent immune response.

The second major task was to establish a set of field sites that would enable us to rigorously test the efficacy of vaccinating robins in reducing West Nile virus transmission. We decided to use 8 sites in the study, consisting of 4 pairs of sites. The two sites in each pair were very close to each other so that factors that might affect the intensity of West Nile virus transmission each year (primarily weather and weather affects on birds and mosquitoes) would

be similar between the two sites. Our four site-pairs were in Foggy Bottom, Baltimore, Bethesda, and Takoma Park (Figure 3).

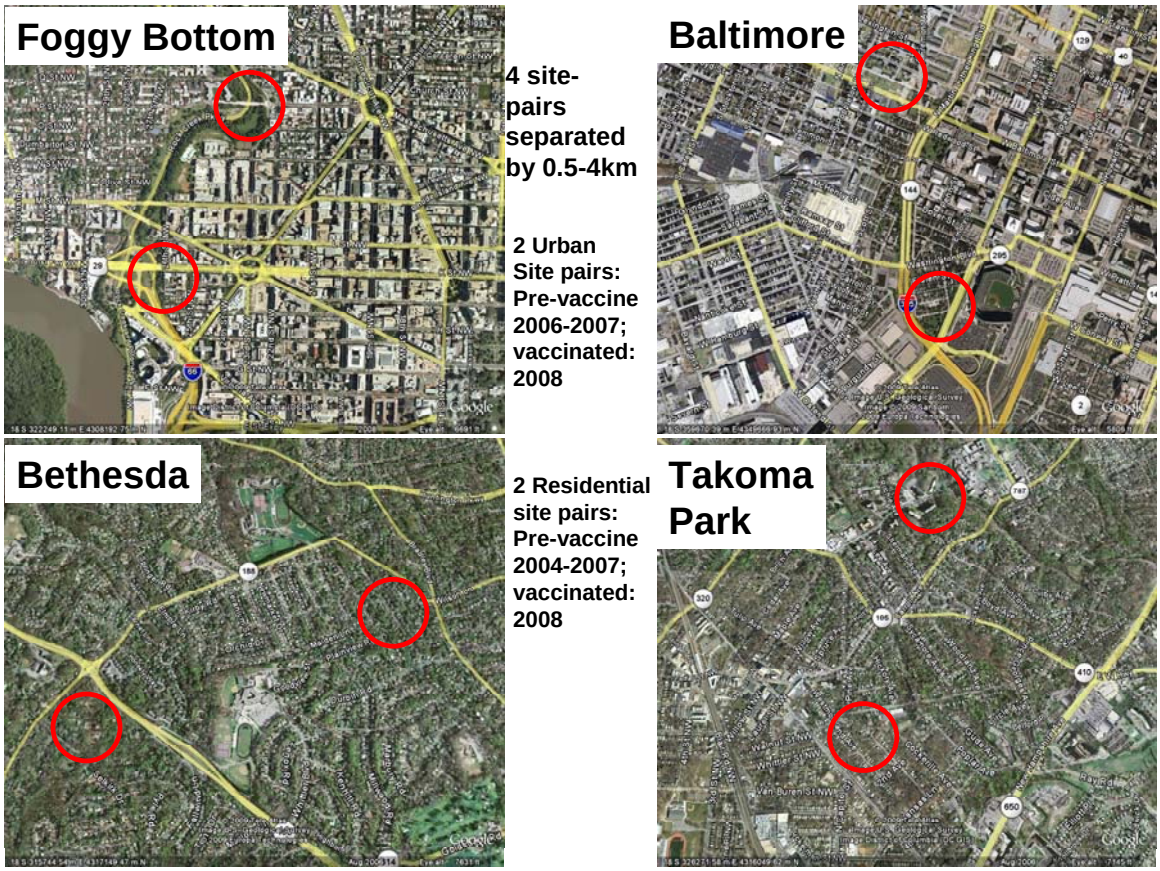


Figure 3. Aerial photos of our four site-pairs in Foggy Bottom (just north of the Watergate Hotel, and 1km north of there), Baltimore (Just west of Camden Yards, and 1.5km north of there), Bethesda (near Wilson and Selkirk, and Wilson and Radnor), and Takoma Park (near Spring Park, and Opal Daniels Park).

We began doing research (catching and censusing birds, and catching mosquitoes, ticks and even mammal in a couple years) at the four Bethesda and Takoma Park sites and one of the Foggy Bottom sites in 2003, and at one of the Baltimore sites in 2004. We added site-pairs to the Baltimore and Foggy Bottom sites in 2006 where we trapped mosquitoes and censused the bird community. We continued these studies in an observational way (i.e. we did not alter any of the sites) through 2007. The two sites in each site pair were similar in mosquito communities (abundance of each of the different mosquito species) (Figure 4), and bird communities (Figure 5).

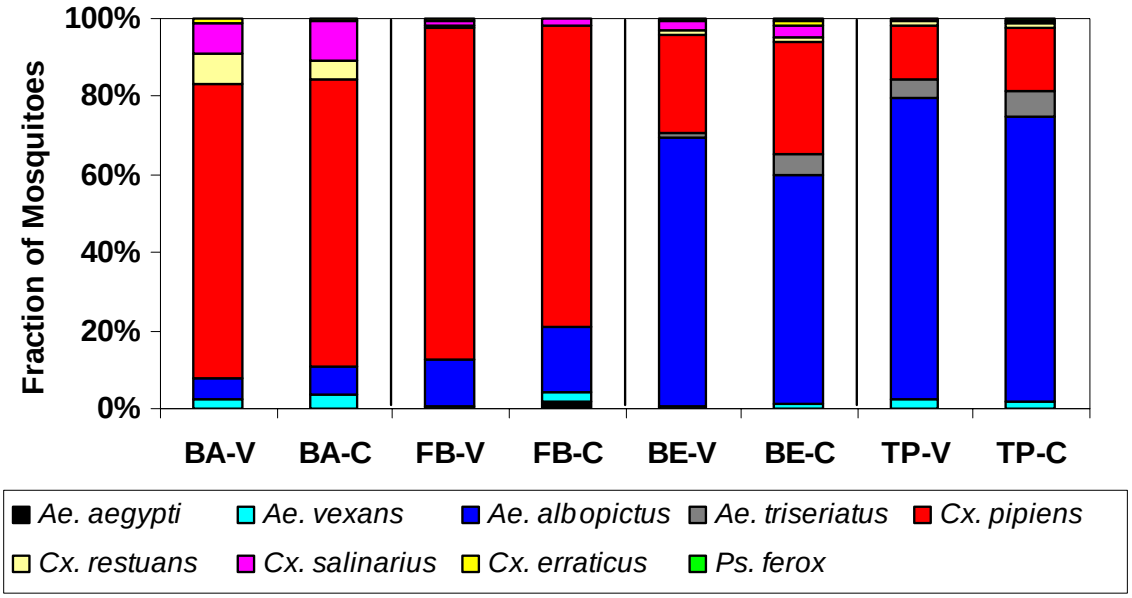


Figure 4. Relative abundance of different mosquito species at each of our 8 sites in four site pairs in Baltimore (BA), Foggy Bottom (FB), Bethesda (BE), and Takoma Park (TP). The site we subsequently chose to vaccinate robins at have a "-V" whereas the "control" sites where we did not vaccinate robins have a "–C" after their name. Not all species have common names, but a few do: The Asian tiger mosquito is in blue (*Ae. albopictus*), the most important West Nile virus mosquito, the northern house mosquito, is in red (*Cx. pipiens*), and the mosquito that can transmit yellow fever and dengue fever is in black (*Ae. aegypti*), which is not very common, thankfully.

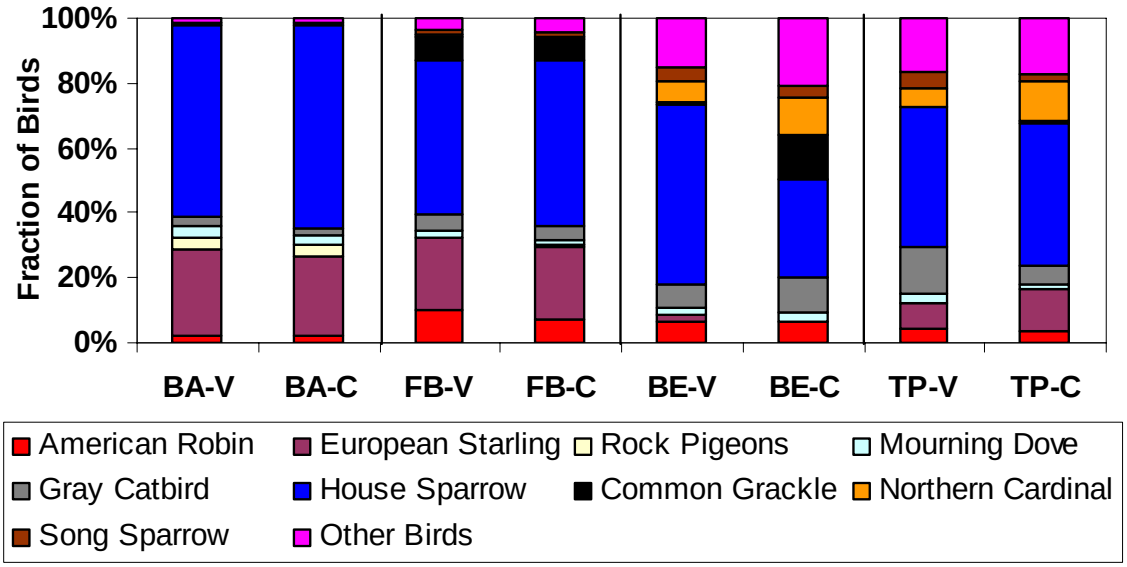


Figure 5. Relative abundance of birds at each of our 8 sites in four site pairs in Baltimore (BA), Foggy Bottom (FB), Bethesda (BE), and Takoma Park (TP). The site we subsequently chose to vaccinate robins at have a "-V" whereas the "control" sites where we did not vaccinate robins have a "–C" after their name.

Then, in 2008, we picked one site of each of the four site pairs to be our "treatment" site (the sites with the "-V" in Figures 4 and 5) where we would vaccinate robins and hope to decrease transmission. At the same time, we continued to measure West Nile virus transmission at the "control" site (the sites with the "–C" in Figures 3 and 4) where we did not vaccinate robins. We chose to vaccinate robins at the site in each site-pair that had higher West Nile virus transmission over the years 2004-2007, because we believed a) this site needed the most help, and b) we could have a larger effect at the higher transmission site. Our "treatment" or "vaccine" sites were the southerly Foggy Bottom, Baltimore, and Takoma Park sites, and the easterly Bethesda sites (Figure 3).

From April-July, 2008, we caught robins for vaccination in two ways: 1) in mist-nets, as we have been doing in our previous work, and 2) by finding robin nests and vaccinating the nestlings just 1-3 days before they left the nest. Using the second method greatly increased the number of birds we were able to vaccinate, because robins are sometimes difficult to catch in mist-nets (after the first hour or two after dawn, they mostly forage on the ground and move by walking, whereas our nets are designed to catch flying birds). This work involved a large amount of tree climbing which was both fun, and sometimes exciting!

In all, we vaccinated 30-75 adult robins and 45-70 juvenile/hatch-year (or young of the year – all these mean the same thing) robins at each of the four sites (Figure 6), which we estimate was 80-94% of the hatch year birds in each study area, and 43-54% of the adults. Our past research indicated that young of the year birds were more important in transmission than adults, so our coverage of juvenile/hatch-year birds was more important.

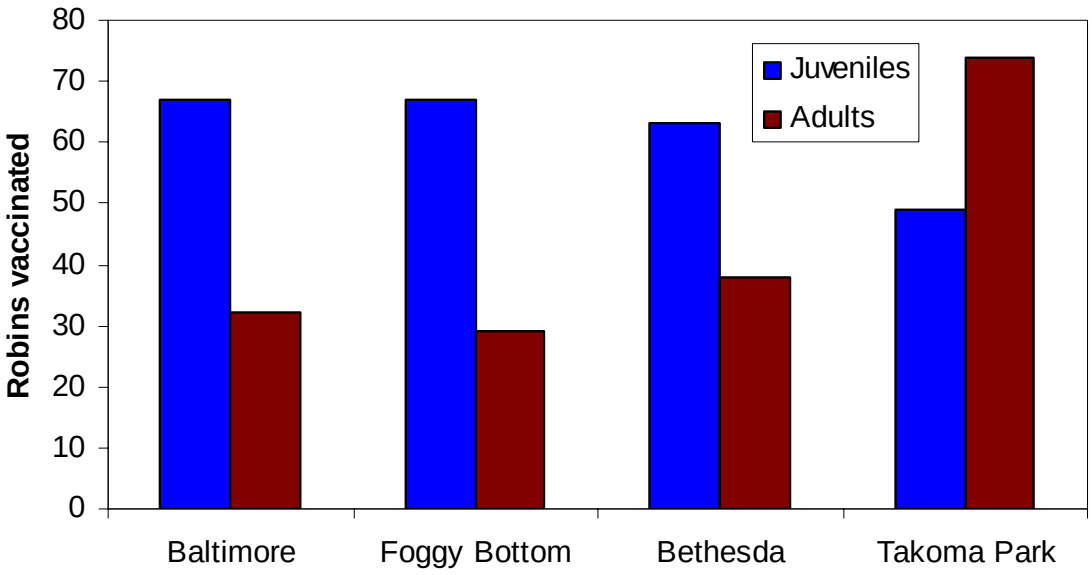


Figure 6. Numbers of robins vaccinated at each of our four "treatment" or "vaccine" sites in 2008.

We measured the intensity of West Nile virus transmission as the fraction or prevalence of mosquitoes that were infected with West Nile virus at each of the 8 sites over the summer (May-October). To determine whether we actually decreased transmission at our "vaccine" sites requires a somewhat sophisticated statistical analysis, but the idea is straight forward and is presented in Figure 7.

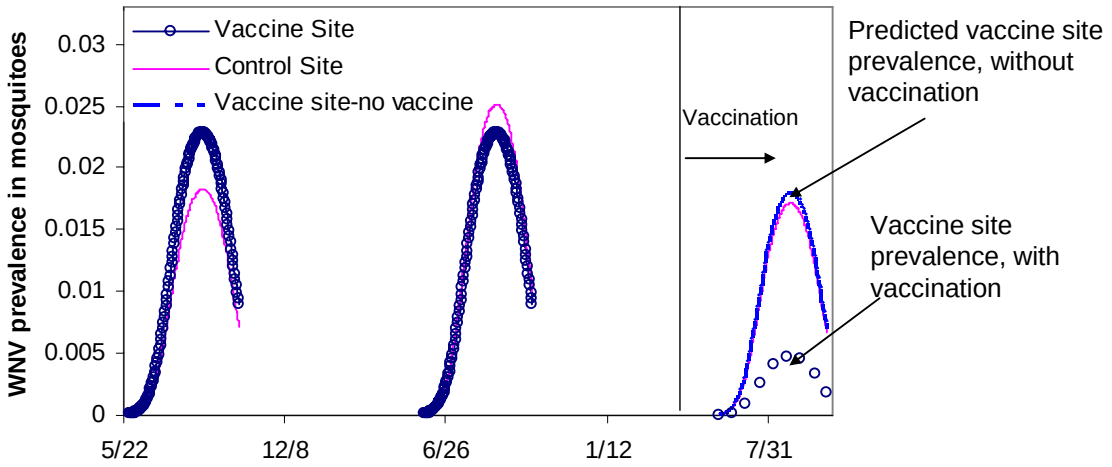
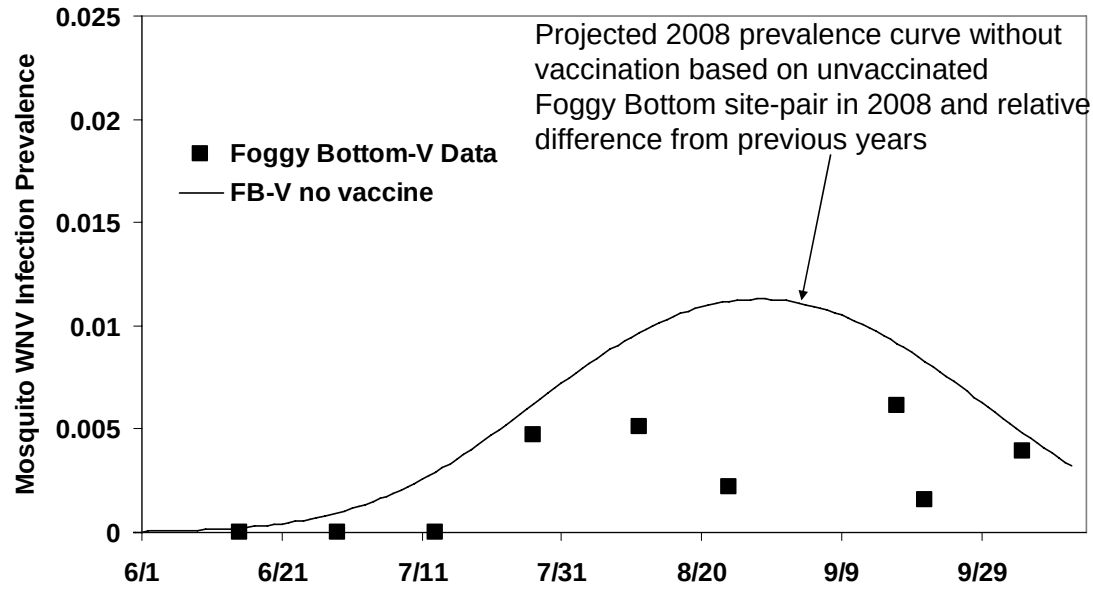
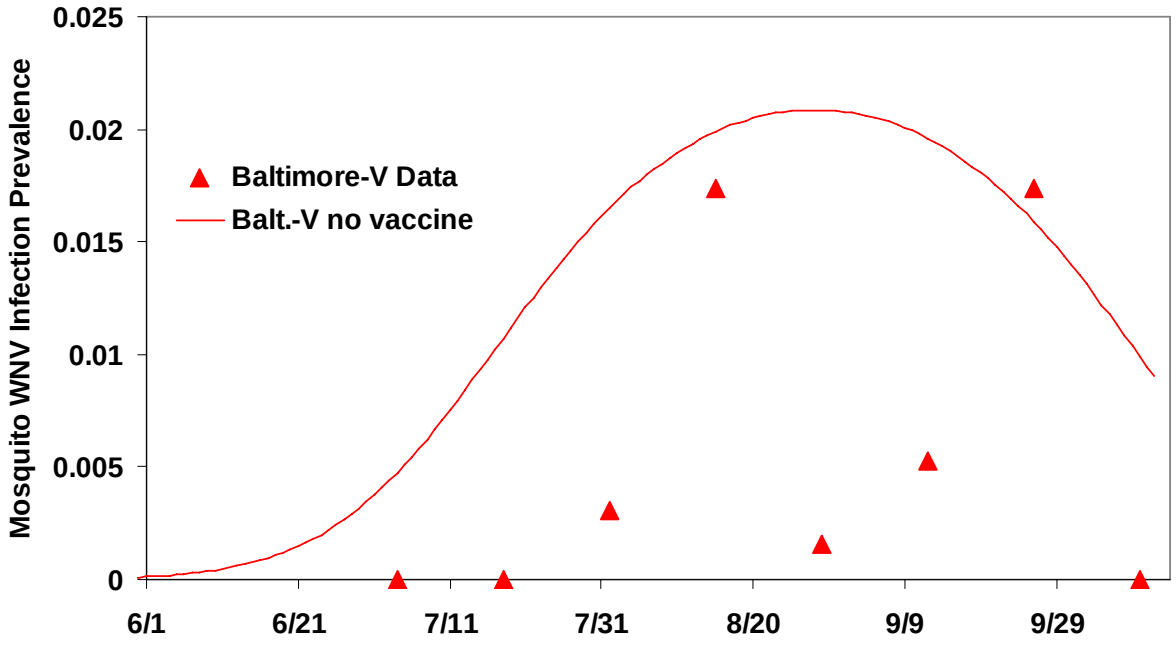


Figure 7. Imaginary data to illustrate the experimental design of our field vaccination study. The graph shows the prevalence (fraction of mosquitoes) infected with West Nile virus on the y-axis, from May-October in each of three years, 2 before vaccinating any birds, and 1 in which we vaccinate robins.

This figure shows imaginary data of the prevalence of West Nile virus in mosquitoes at two nearby sites (i.e. 1 site-pair) over each of three years (we can pretend the years are 2006, 2007, and 2008). The two sites differ a little bit from year to year, but not too much (because they are very near to each other and have similar bird and mosquito communities). In the 3rd year, we can predict what the prevalence of West Nile virus would be in mosquitoes at the vaccine site if we did not vaccinate any birds based on what transmission is like at the "control" site in 2008, and the average difference, if any, in previous years (in the imaginary data in Figure 7, the "vaccine" site was a little higher, on average, over the previous two years). We can compare this prediction or expectation to what we actually measure at the "vaccine" site in 2008 when we vaccinate robins. If we were successful, the prevalence of West Nile virus in mosquitoes in 2008 at the "vaccine" site would be much lower than what we expected if we hadn't vaccinated any robins.

The results of our field experiment are shown in Figure 8.


Mosquito WNV Infection Prevalence
0.025
0.02
0.015
0.01
0.005
0
6/1
6/21
7/11
7/31
8/20
9/9
9/29
Foggy Bottom-V Data
FB-V no vaccine
Projected 2008 prevalence curve without vaccination based on unvaccinated Foggy Bottom site-pair in 2008 and relative difference from previous years


Mosquito WNV Infection Prevalence
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Baltimore-V Data
Balt.-V no vaccine

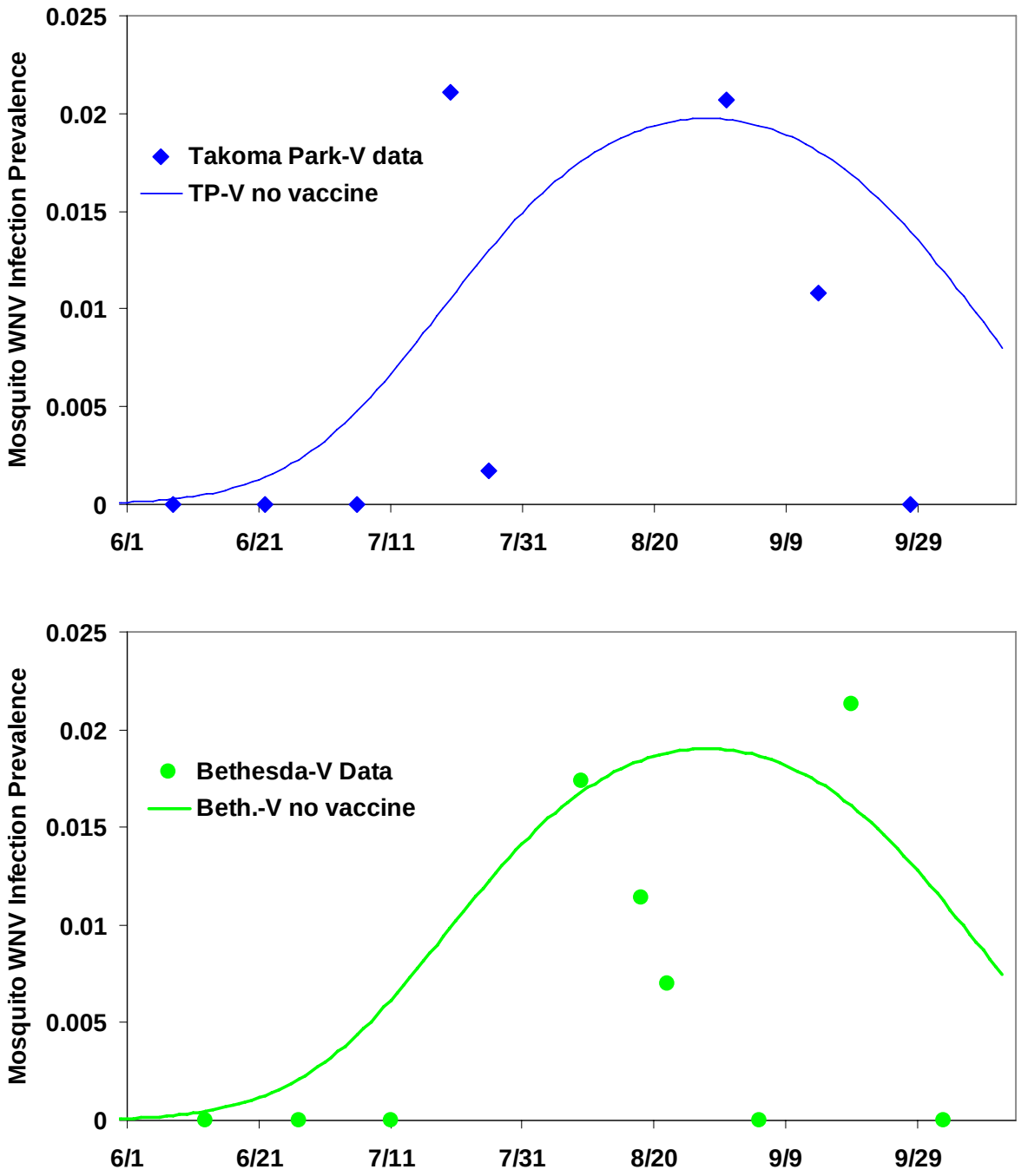

Figure 8. Results of robin vaccination study at the four sites. Y-axis is the fraction/prevalence of West Nile virus in mosquitoes, and X-axis is the date. The line shows the prevalence we would have expected had we <u>not</u> vaccinated robins (the upper blue line in the third year in Figure 6) based on data from the control site (which is not shown because it would make the graph difficult to read) and differences between the two sites in each site-pair over the period 2004-2007. The symbols show the actual data – the observed prevalence of West Nile virus at the vaccine site, which is often (but not always) lower than the "expected" prevalence, indicating that vaccination reduced the fraction of mosquitoes

that were infected. In all, we trapped and tested 76,544 mosquitoes at the 8 sites over the five years.

The statistical analysis, and a rough glance at these figures indicate that vaccinating robins substantially reduced the fraction of mosquitoes that were infected with West Nile virus. The actual amount was approximately 64%, but, as is clear from Figure 8, the effect at some sites was a little higher and at some sites a little lower. Nonetheless, overall, the study was a major success, and I confess a little surprising! I had worried that given how much birds fly around that we might not have been able to vaccinate a large enough fraction of the robin population at our sites. But, it appears that we did! The fact that we were able to reduce West Nile virus transmission by 64% by hand vaccinating birds in a relatively small area is very promising for using this strategy on a larger scale. However, it would require the development of a vaccine that would be effective when ingested (we had to vaccinate the robins by injection), and then it would be necessary to find a way to get robins to eat the vaccine (worms with vaccine?). Clearly we are a ways off before this can be a viable public health strategy, but it is encouraging nonetheless!

West Nile virus transmission in mosquitoes and birds along our urbanization gradient

We also performed our mosquito and bird studies at 10 other sites in 2008. At each of these sites we:

1) performed a census or survey of the bird community each month, to determine which birds were present at each site and their relative abundance
2) captured, banded, and took blood samples from birds which we tested for West Nile virus antibodies
3) trapped mosquitoes using dry ice (CO_2)-baited CDC light traps, and CDC gravid traps (for egg-laying mosquitoes) baited with organically rich water

Over the past six months the NY State Dept. of Health has been testing our samples:

1) the mosquitoes we trapped were tested for West Nile virus
2) the birds were tested for antibodies to West Nile virus

In total, between May and October, 2008, we caught 5,864 resident birds, 47,809 mosquitoes, and counted 8,746 birds on our censuses. Below I describe the data from each of these components and some of the preliminary conclusions that we can draw from these data.

Mosquito Abundance and West Nile virus Prevalence

Figure 9 shows the abundance of two different types (genera) of mosquitoes and the fraction that were infected with West Nile virus from each of the sites we trapped mosquitoes in 2007. *Culex* mosquitoes bite mostly birds, but sometimes feed on mammals (including humans). In contrast, *Aedes* mosquitoes feed primarily on mammals (including humans). The fraction of mosquitoes that are infected with West Nile virus is labeled "*Culex* IP" which denotes the *Culex* Infection Prevalence. *Culex* mosquitoes are the primary mosquitoes for transmitting West Nile virus.

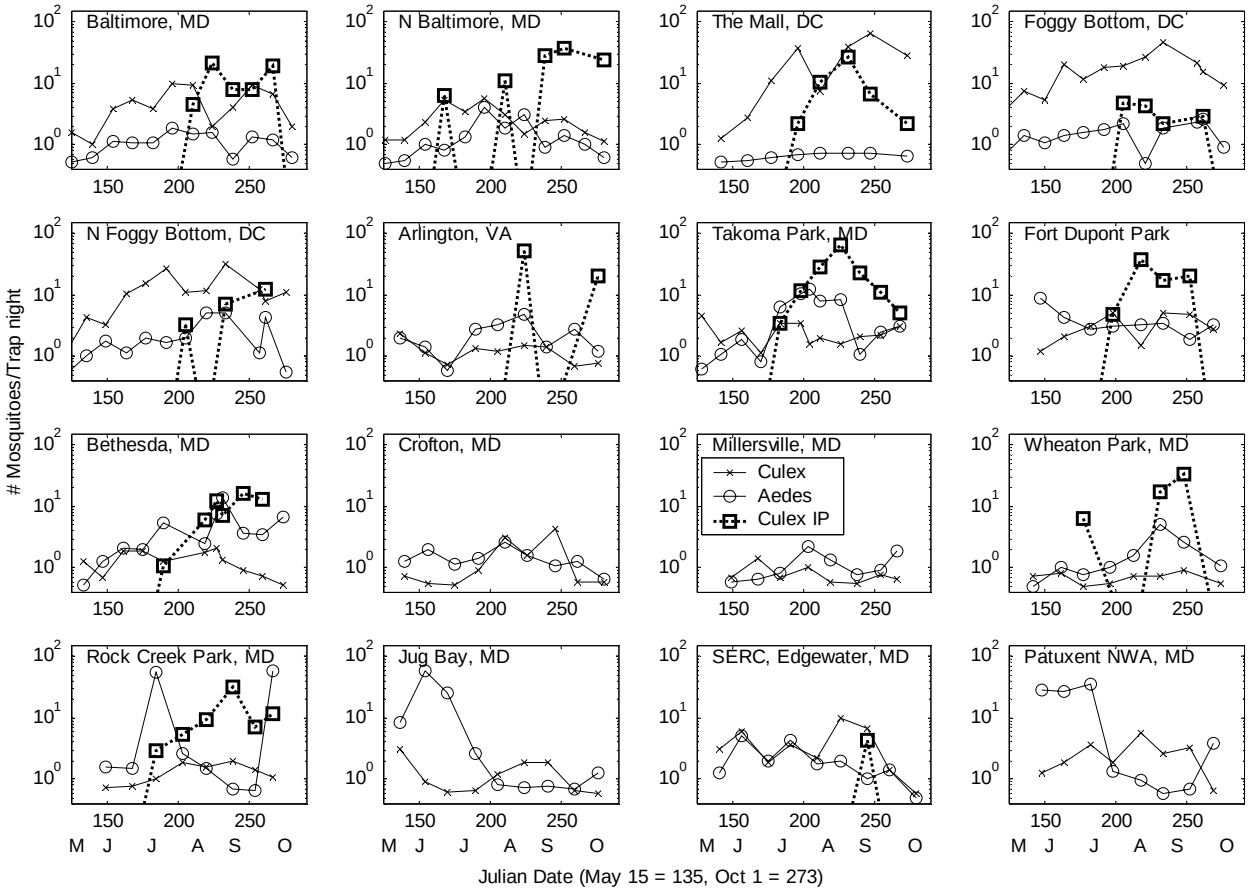

Figure 9. 2008 *Abundance of host-seeking Culex and Aedes mosquitoes and Culex WNV infection prevalence (IP) on a log scale at 16 sites arranged from most urban (upper left) to most forested (bottom right), going across and then down. No infected mosquitoes were trapped at Crofton, Millersville, Jug Bay, or Patuxent NWA in 2008.*

The Infection Prevalence (IP) is a measure that mosquito control people and scientists use to describe the fraction of mosquitoes that are infected with West Nile virus. It is equal to 1000 times the fraction of mosquitoes that are infected with West Nile virus. People use this measure because the fraction of mosquitoes infected with West Nile virus (and other viruses) is usually very low (1 in a 1000), so instead of having to write 0.001 or 0.002, they just write 1 or 2. One last thing that is important when looking at Figure 1 is that the y-axes (the vertical dimension) are drawn on a logarithmic scale, so that each little tick mark is a large jump. On the y-axis, $10^2 = 100$, whereas $10^1 = 10$, and $10^0 = 1$. So, the graphs show a span of 100-fold.

Here's what these data tell us:

1) First, 2008 was a very intense year for West Nile virus activity. The number of human cases in 2008 in Maryland (14) was similar to 2004 (16 cases), another intense year. Our mosquito data show both quite high infection prevalences in mosquitoes, and moderately high mosquito abundances (Figure 8).
2) One of the most interesting things about West Nile virus transmission in 2008 is that we detected infection in mosquitoes in all our urban sites, many residential sites, all of our forested parks, and even one of our forested sites (SERC), where we'd never detected

West Nile virus in mosquitoes before. We had substantial transmission at Rock Creek park and Wheaton regional park, and very high infection prevalences at Fort Dupont Park, in Takoma Park, and at Baltimore.

West Nile virus antibody prevalence in Birds

Figure 10 shows the antibody prevalence of hatch-year birds (the fraction of birds with antibodies against West Nile virus, meaning they were bitten by an infected mosquito and survived the infection) across the 14 sites where we sampled birds from May to October in 2008. Figure 11 shows the same data from 2007 which I hadn't included in previous reports because the testing wasn't done yet.

Here's what these data tell us:

3) First, the data from 2008 confirm the mosquito results above, in that it was a very intense year for West Nile virus activity. Two of our sites that are good barometers for transmission because we sample cardinals (a very good indicator species) in good numbers are Takoma Park, and Fort Dupont Park. At these two sites, essentially all hatch year N. cardinals (NOCA) had been exposed to West Nile virus by the end of the season (the line for cardinals is ~1 by September). In contrast, in 2007 (Figure 10), only 40-60% of cardinals at these two sites had been exposed by the end of the transmission season. Exposure of birds at several other sites was also high in 2008, although small sample sizes of birds caught in a given month at a site sometimes prevent us from measuring exposure accurately. For example, despite finding low levels of West Nile virus infected mosquitoes at SERC in 2008, we didn't find any hatch year birds that had antibodies in 2008. This is likely because it's challenging to catch a good sample of hatch year cardinals at SERC where they are less common than Takoma Park, Fort Dupont Park, Bethesda, or Rock Creek Park.
4) The gradient of West Nile virus transmission from highest in urban areas to lowest or nearly absent in forested areas was again true in 2008 and 2007. The easiest way to see this is to compare exposure of N. cardinals at Takoma Park (a residential area), Fort Dupont Park (a fragmented park surrounded by intensive development), Bethesda, Crofton, and Millersville (three residential areas with a larger number of trees than Takoma Park), Rock Creek Park (in Rockville, MD; a park in a rural area), SERC (a mostly forested area with a small set of research buildings), and Patuxent National Wildlife Refuge (a mostly forested area where we did catch a single antibody positive hatch year bird in 2008, our first ever at this site). Exposure was highest (near 100%) at Takoma Park and Fort Dupont Park where mosquito infection rates were also the highest, and were much lower at the more forested residential areas (Bethesda, Crofton, Millersville), park in a rural area (Rock Creek Park), and actual forested areas (SERC and Patuxent).

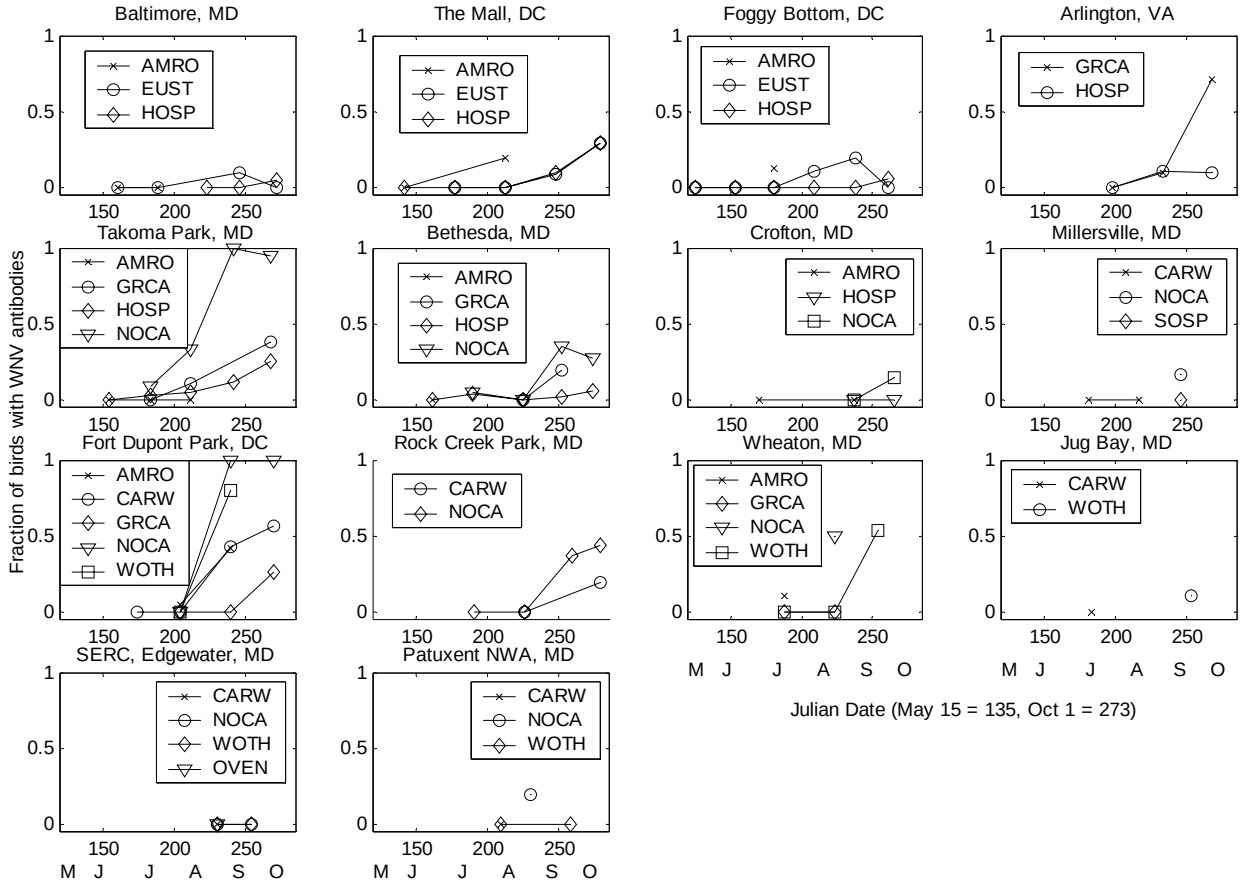

Figure 10. *West Nile virus antibody prevalence (fraction of birds with antibodies) for hatch year birds at our 14 sites in 2008. Each point represents an average of 8.6 (range 4-49) birds. Sites are arranged from most urban (upper left) to most forested (bottom right), going across and then down. Species abbreviations: ACFL - Acadian Flycatcher, AMRO American Robin, CARW – Carolina Wren, CACH – Carolina Chickadee, ETTI – Tufted Titmouse, EUST – European Starling, GRCA – Grey Catbird, HOSP – House Sparrow, NOCA – Northern Cardinal, NOMO – Northern Mockingbird, OVEN – Ovenbird, REVI – Red-eyed vireo, RODO – Rock Dove, WOTH – Wood Thrush.*

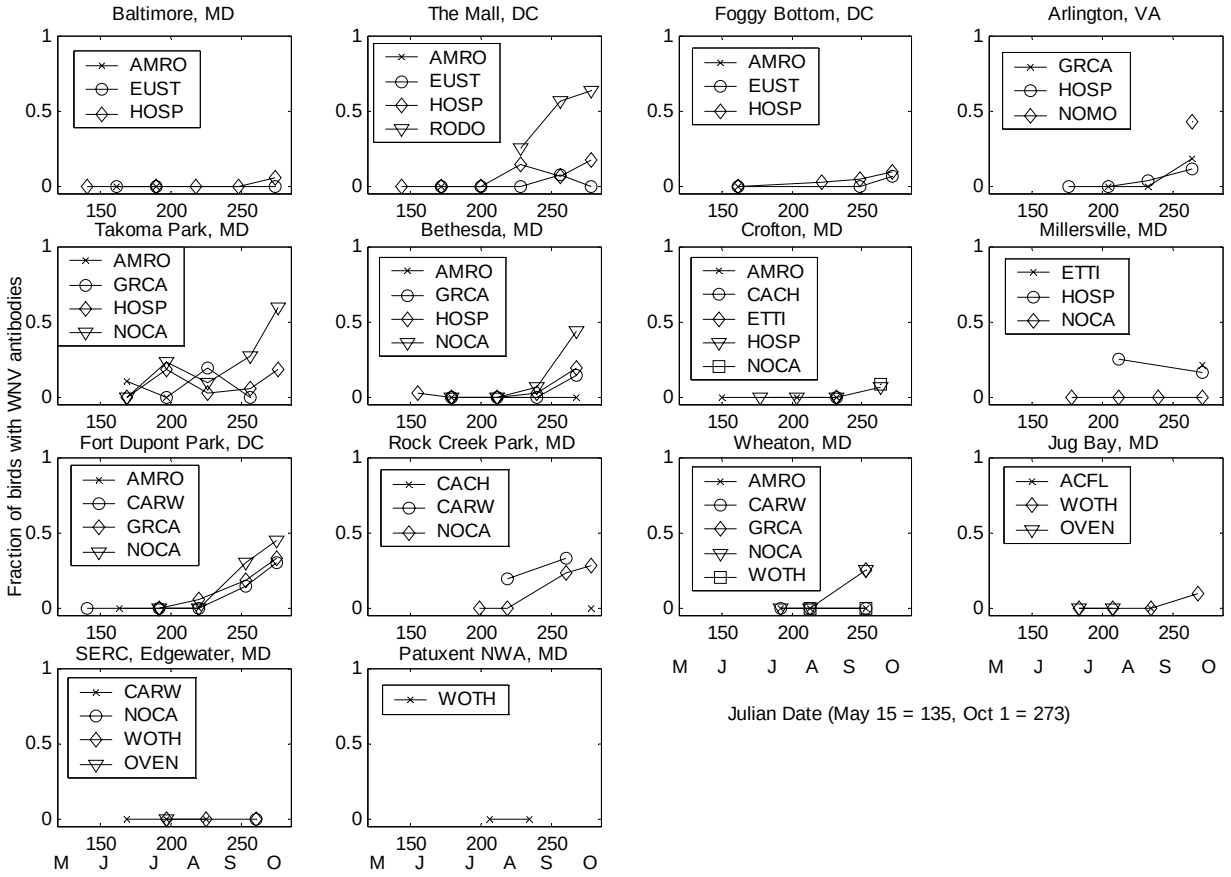

Figure 11. *West Nile virus antibody prevalence (fraction of birds with antibodies) for hatch year birds at our 14 sites in 2007. Each point represents an average of 8.8 (range 4-51) birds. Sites are arranged from most urban (upper left) to most forested (bottom right), going across and then down. Species abbreviations: ACFL - Acadian Flycatcher, AMRO American Robin, CARW – Carolina Wren, CACH – Carolina Chickadee, ETTI – Tufted Titmouse, EUST – European Starling, GRCA – Grey Catbird, HOSP – House Sparrow, NOCA – Northern Cardinal, NOMO – Northern Mockingbird, OVEN – Ovenbird, REVI – Red-eyed vireo, RODO – Rock Dove, WOTH – Wood Thrush.*

Survival of *Culex pipiens* mosquitoes at three sites in 2009

The survival of mosquitoes is a critical factor in virus transmission because for most pathogens the mosquito must bite one infected host and then survive long enough for the virus or bacteria or malaria parasite to develop and replicate inside the mosquito and infect the salivary glands of the mosquito. Until the virus reaches the salivary glands, it won't be transmitted when the mosquito feeds next. Thus, if a mosquito doesn't live long enough for the virus to reach the salivary glands, then the virus won't be transmitted. Knowing how long mosquitoes survive, which can differ substantially between mosquito species, is also important for control, because it determines how large of an effect spraying will have on transmission.

So, in 2007 Christy Johnson, a Master's student working on the project, performed a pilot study to measure the survival of *Culex pipiens* mosquitoes (a species for which no one has ever measured survival in the field before) in Takoma Park. In July 2008 she and our team measured the survival of mosquitoes at 3 sites, Takoma Park, Fort Dupont Park, and Baltimore. To

measure survival, we dusted a few hundred mosquitoes from each of the sites with a UV-florescent dust, and then trapped huge numbers of mosquitoes over the next 15 days to measure how long marked mosquitoes survived at the sites.

We were most successful in marking mosquitoes at Takoma Park (1000 mosquitoes), and less so at Baltimore (only 200 mosquitoes), with Fort Dupont being intermediate, and as a result, our results are most robust at Takoma Park. Figures 12, 13 and 14 show the results of these three studies: they show the number of marked mosquitoes caught in traps on each day after the initial marking. The decay in marked mosquitoes results from mortality and statistical techniques can be used to estimate the survival and lifespan of mosquitoes from these data.

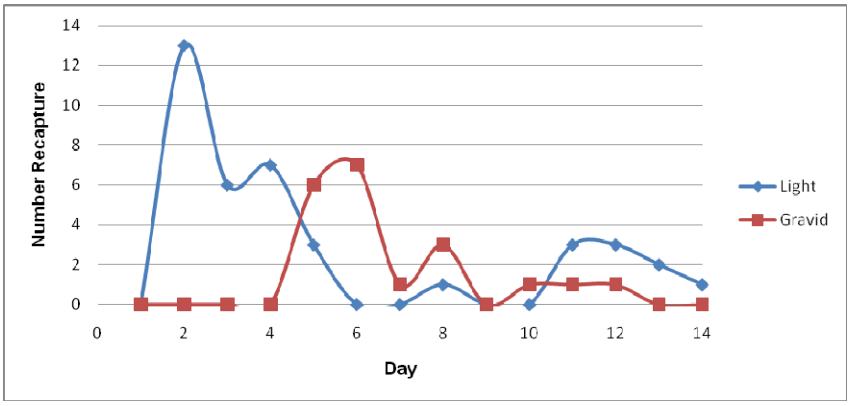

Figure 12. *Numbers of recaptured marked Culex pipiens mosquitoes in CDC light (host seeking traps) and gravid (egg-laying) traps at Takoma Park in 2009.*

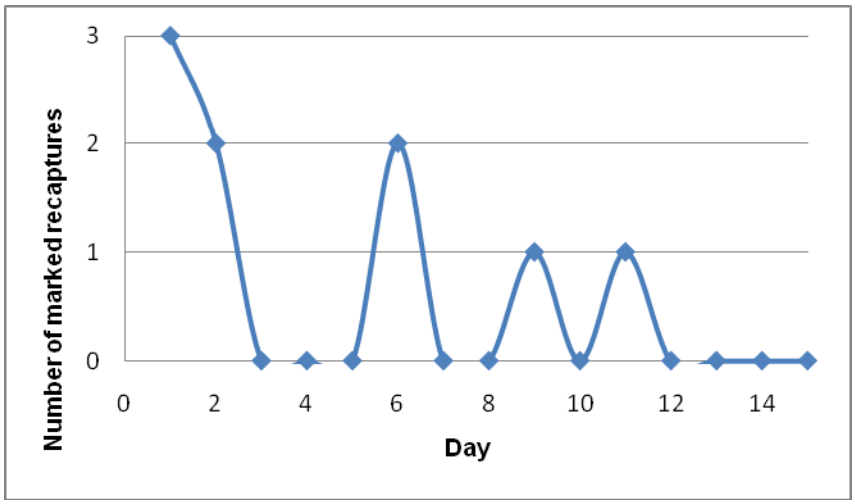

Figure 13. *Numbers of recaptured marked Culex pipiens mosquitoes in CDC light (host seeking traps) traps at Fort Dupont in 2009.*

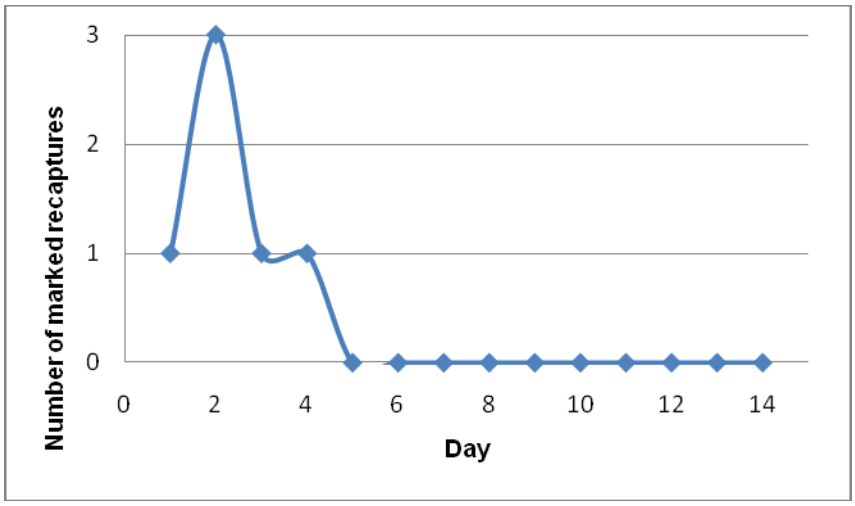

Figure 14. *Numbers of recaptured marked Culex pipiens mosquitoes in CDC light (host seeking traps) traps at Baltimore in 2009.*

Our statistical analyses of these data allowed us to calculate the survival (and probability of recapturing a *Culex pipiens* mosquito) at these three sites, although the estimates from Takoma Park are the most reliable (Table 1). They suggest that these mosquitoes live, on average for about a week in Takoma Park, but less at the other two sites.

Table 1. *Survival rates (survival ± SE) of Culex pipiens females.*

Site, Yr	*# Marked*	*# Recap-tured*	*% Recap-tured*	*Daily survival*	*Lifespan (days)*
Takoma Park 2008	223	17	7.6	0.835±0.06a	6.06
Takoma Park 2009	1000	58	5.8	0.860±0.03a	7.14
Baltimore 2009	200	6	3	0.606±0.05a	2.54
Fort Dupont 2009	766	9	1.2	0.517±0.13b	2.07

Daily Survival estimates with same letters are not statistically different.

In 2009, we aim to replicate the survival studies at Fort Dupont and Baltimore, and also estimate survival at SERC, and possibly in Bethesda, MD.

Remaining questions

The big outstanding question is what makes some years intense West Nile virus years (2008, 2004) and others relatively mild (2007, 2005). Our hypothesis is that climate (temperature and rainfall) influence mosquito abundance and the replication rate of the virus in mosquitoes, and that these factors in turn determine the intensity of transmission. Testing this hypothesis requires several years of data over which climate varies, and we are approaching a sufficient dataset to address this important question. This is one of the key goals for this and next year's field season – to continue building our multi-year dataset on West Nile virus transmission to understand year to year variation and the impact of climate.

In addition, we are adding a few new components to the study each year. In 2010, we will be studying the effect of West Nile virus on the survival of American robins and Northern

Cardinals by placing radio transmitters on birds at four sites: Takoma Park, Fort Dupont Park, Foggy Bottom, and Rock Creek park.

Thanks you again for your help in making our research possible. We hope that you will allow us to continue to use your property for our study.